CHROMOSOME NUMBERS AND CHARACTERISTICS OF A PROGENY OF A TRIPLOID GUAVA CLONE

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INTRODUCTION

Psidium guajava L. was found to be a diploid with 22 somatic chromosomes (Darlington and Janaki Ammal, 1945; Atchison, 1947; Kumar and Ranade, 1952; D'Cruz and Rao, 1962; Seth, 1963; Hirano and Nakasone, 1969a).

Polyploid plants are not common, but have been found in orchards in India. A plant with 33 chromosomes was described (Kumar and Ranade, 1952) and its meiosis was found to be highly abnormal. The high percentage of trivalents during its first division suggested autotriploidy and explained the absence of seeds in the fruits.

The triploid 'Indonesian Seedless' guava clone (Hirano and Nakasone, 1969a) was introduced into Hawaii from India. The plant is vigorous, grows upright, and the fruits are white-fleshed, sweet (Whitman, 1962) and usually contain a few seeds.

Two seedlings derived from an open-pollinated triploid guava clone were found to have 21 and 30 chromosomes (D'Cruz and Rao, 1962). They were believed to have resulted from a cross between the triploid seed parent and a diploid guava plant. In another study, three open-pollinated seedlings from the 'Indonesian Seedless' clone showed somatic chromosome numbers of 21, 24, and 25 (Hirano and Nakasone, 1969a). They were also believed to have resulted from a 3n X 2n cross.

The present study is an attempt to continue the investigation of the chromosome numbers of more seedlings of the 'Indonesian Seedless' guava and to relate their chromosome numbers to morphological differences and reproductive behavior.

REVIEW OF LITERATURE

Origin and Distribution

The guava originated in Central America where it is found in the wild as well as in cultivation. After the discovery of the New World, it spread rapidly across the Pacific to Asia. Today, it is found in many countries of the tropics, South Africa, southern Florida, southern California, and parts of the Mediterranean.

It is adapted over a wide range of climatic and soil conditions and grows from sea level to 5,000 feet. The plant can tolerate temporary waterlogging and high temperatures but is susceptible to frost. The seeds are spread easily by birds and other animals and the plants are hardy, drought resistant and vigorous, to the extent that they are sometimes considered weeds in places like southern Florida, Cuba, Hawaii and Fiji. (Ruehle, 1948; Cobley, 1956; Purseglove, 1968.)

Botanical Description

The plant is a shallow-rooted shrub or small, spreading tree ranging from 6 to 30 feet in height. Branching usually occurs near the base and suckers are often produced from the roots. The trunk is relatively smooth and thin but the bark is scaly and multi-colored. Young stems are green and angular. Leaves are opposite, oblong-elliptic or oval, 8 to 15 cm. long with prominent veins and a soft down underneath. Petioles are short and grooved above. (Bailey, 1947; Ruehle, 1948; Cobley, 1956; Neal, 1965; Purseglove, 1968.)

Flowers are axillary, usually single, fragrant and about 2.5 cm. across. There are 4 to 6 free petals which are white and obovate.

Stamens are white and numerous with pale yellow anthers. The single style is central and has a knob-shaped stigma. (Bailey, 1947; Ruehle, 1948; Cobley, 1956; Neal, 1965; Purseglove, 1968.)

The ovary is inferior and consists of 4 or 5 united carpels, each containing numerous ovules arranged in axile placentation. The fruit is a round to oblong, ovate, globose or pyriform berry about 2.5 to 6.5 cm. in diameter. It contains numerous small hard seeds borne in 2 to 7, commonly 4, locules. The flesh may be white, yellow, pink or salmon-colored and is derived from the ovary wall and the placenta. (Bailey, 1947; Ruehle, 1948; Cobley, 1956; Neal, 1965; Purseglove, 1968.)

Floral Biology

In Hawaii, the main guava crop ripens from May through August while a smaller second crop is produced during the winter season (Hamilton and Seagrave-Smith, 1954). These fruiting periods indicate that flowering occurs between February and April for the main crop and between October and December for the second crop. The two flowering seasons in India are from September to October and from January to March, each lasting about 30 to 60 days (Balasubrahmanyam, 1959).

The flower buds show a cracking of the calyx nearly 24 hours before anthesis. In India, anthesis occurs between 5:00 a.m. and 7:00 a.m., depending on the clone (Balasubrahmanyam, 1959). Floral anthesis in Hawaii starts around 6:00 a.m., and by 8:00 a.m., all the flowers are opened. The period of stigma receptivity appeared to be limited to approximately 48 hours after anthesis, while 48 and 72 hour-old pollen gave sixty and twenty percent fruit set respectively (Hirano and Nakasone, 1969b).

Pollination and Compatibility

Both self- and cross-pollinations occur in guavas. Isolated trees often set satisfactory crops of fruits without cross-pollination (Hamilton and Seagrave-Smith, 1954). The flowers are visited by bees and other insects. Using the red mesocarp color as a marker, natural cross-pollination has been shown to be about 35 percent (Purseglove, 1968).

Studies in India showed cross-incompatibility among several clones (Seth, 1960). In Hawaii, incompatibility was observed in crosses between the triploid 'Indonesian Seedless' and some diploid clones (Ito and Nakasone, 1968). The same study also showed varying degrees of incompatibility in crosses between certain diploid clones.

Polyploidy

The first report of polyploidy in *P. guajava* (Kumar and Ranade, 1952) described a plant with 33 chromosomes.

Another shy bearing, seedless guava clone was reported to be a diploid (Seth, 1959) with about 68 percent of the microspores having normal meiotic division and the rest showing laggards and bridging. However, certain discrepancies in the report led to a re-examination of the plant which subsequently showed 33 somatic chromosomes and a high number of trivalents per PMC (Majumder and Singh, 1964).

The seedless guava reported by D'Cruz and Rao (1962) is also a triploid which exhibited mostly trivalents with some bivalents and univalents during diakinesis and metaphase-I. The distribution of the chromosomes to the two poles at anaphase-I varied from 12 to 21 and there was no visible difference in the size of the tetrads formed. The plant had an erect habit, oblong leaves 15.2 cm. long and 7.8 cm. broad, 8 petals per flower, and oval fruits. The chromosome complement consisted of 18 with median and 15 with sub-median primary constrictions, and 3 with secondary constrictions.

The triploid 'Indonesian Seedless' clone exhibited pollen germination of 2.4 percent (Hirano and Nakasone, 1969b). Without cross-pollination it tends to be a shy bearer with completely seedless and irregular fruits (Whitman, 1962). Fruits of the plants grown at the Poamoho Experimental Farm (Oahu, Hawaii) were analyzed (Nakasone, Hamilton, and Ito, 1967) and found to have the following characteristics:

Fruit weight	6.2 oz.
Cavity diameter	4.3 cm.
Total diameter	7.3 cm.
Seed content (by weight)	0.7 %
Soluble solids	12.5 %
Total acidity (in terms of citric acid) .	0.4 %

A tetraploid plant, discovered among seedlings of the 'Safeda' clone, was compared with the diploid and triploid plants (Shanker, et al., 1964). Its growth was found to be more vigorous and the size of the various vegetative parts distinctly larger. Young shoots were pronouncedly angular and winged. The leaves were broader, thicker, darker green and the pedicels were longer. The fruits were sweeter and were either round and seeded or irregular and seedless.

Another tetraploid plant was reported to have flowered more or less throughout the year (Naithani and Srivastava, 1966). Its meiosis was found to be highly irregular and the high percentage of quadrivalents during diakinesis and metaphase-I suggested autotetraploidy. The plant was assumed to have resulted either from the union of two unreduced gametes of the diploid plant or from somatic doubling. According to

Naithani and Srivastava (1966), triploid fruits are inferior to diploid fruits but somewhat better than the tetraploid fruits in size and taste. It was concluded that polyploidy did not play an important role in the improvement of quality in the guava fruits.

On the other hand, polyploidy has played an important role in the improvement of many cultivated plants. Among the orchids, triploidy appears to be the most desirable level for cut flower production, especially in Cattleyas and Cymbidiums (Kamemoto, Tanaka, and Kosaki, 1961). Triploidy is also a desirable level for sugar beet production (Allard, 1960). In watermelons, the yield per unit area from triploid plants was better and in some cases doubled that of the diploid parent (Kihara, 1951).

In the case of commercial bananas, triploidy is associated with seedlessness, vigor, and size of the plant and the fruit (Allard, 1960). Many American apple and European pear clones are triploids. The reduction of fertility in triploid apples and pears is an advantage for the fewer fruits produced are larger and more regularly borne (Darlington, 1963). Sterility in the triploid Japanese flowering cherry is also considered an advantage. Triploid selections of tea and mulberry and natural triploid varieties of chrysanthemums are also grown in Japan (Elliott, 1958).

The cultivated potato varieties grown in the United States and Europe are mostly tetraploids (Allard, 1960). Tetraploid clover, rye, turnip, dill, spinach, radish, apples and grapes are also grown commercially (Elliott, 1958).

Autopolyploids could be characterized by having an increased individual cell size, slower growth rate, thicker leaves, larger and fewer flowers, larger fruits and later flowering habit than their diploid counterparts (Elliott, 1958). It is an established fact that, on the average, races within the same species having different chromosome numbers show a very marked positive correlation between chromosome number and gigas characters (Müntzing, 1936).

Aneuploidy

A *Datura stramonium* plant with a rounded instead of a normally elongated pod was found in 1915, growing in the botanical gardens at Storrs, Connecticut. It was hence identified as the "Globe" type. Crosses using this as a parent produced more off-type progeny than normal. It was not until 1920 that Belling proposed an explanation for the morphological variations. (Avery, Satina, and Rietsema, 1959).

Examination of the first division of the PMC's of the off-type plants showed the presence of 12 and 13 chromosomes instead of the constant 12 chromosomes in the gametes of the normal plants. This explained the presence of 25 somatic chromosomes in the "mutants" instead of the normal 24. The 13-chromosome gamete was probably due to the duplication of one of the regular 12. (Blakeslee, Belling, and Farnham, 1920.)

Since then, extensive studies have been made on the genetics and cytology of *D. stramonium*, revealing many more morphologically different types with the duplication of one or more of the 24 chromosomes and exchanges of materials between different arms of chromosomes (Avery, Satina, and Rietsema, 1959; Burnham, 1962).

Aneuploids could be produced from tetraploids, triploids, or diploids which have a tendency for abnormal meiosis (Burnham, 1962). In *D. stramonium*, almost one-half of the plants of a progeny of a 3n X 2n cross were found to be trisomics (Avery, Satina, and Rietsema, 1959). In Gladiolus (2n=30), however, the progeny of a triploid X diploid cross showed a wide range of distribution and variation in their chromosome complement, ranging from 33 to 75 chromosomes (Jones and Bamford, 1942). However, the majority of the plants were either triploids (3n=45) or tetraploids (4n=60).

In tomatoes the F_1 progeny of a triploid X diploid cross did not have a binomial distribution (Lesley, 1928). The chromosome complement ranged from 24, the diploid somatic number, to 27, with the mode at 25 chromosomes. But, the counts were made from only about half of the seedling population.

Detailed studies of aneuploidy, especially of monosomics and trisomics, have been made on many plants. Among them are the possible phenotypic effects of duplication for whole chromosomes or arms of chromosomes and the determination of linkage groups in Datura stramonium, using primary, secondary, and tertiary trisomics (Burnham, 1962). Rick and Barton (1954) cytologically identified 11 of the 12 primary trisomics of Lycopersicon esculentum cv. 'San Marzano', and using genetic tests, found the relationship between several chromosomes, genes and linkage groups. Twenty-four monosomic types of Tabacum were identified and studied (Clausen and Cameron, 1944), resulting in the location of 18 genes in nine chromosomes. Janick, Mahoney, and Pfahler (1959) classified the six primary trisomics of Spinacia oleracea derived from triploid pistillate X diploid staminate crosses. They also found that the extra chromosome was transmitted through the four pistillate trisomics studied and located the gene for sex determination on one of the chromosomes.

In guavas, two seedlings derived from an open-pollinated triploid clone were found to be aneuploids with somatic chromosome numbers of 21 and 30 (D'Cruz and Rao, 1962). The monosomic plant had a spreading habit, with leaves 11.0 cm. long and 5.1 cm. broad, tapering towards the apex. It had 11 petals per flower and round fruits. The chromosome complement consisted of 11 with median and 10 with sub-median primary constrictions and 2 with secondary constrictions. Its meiotic behavior exhibited 10 bivalents and 1 univalent at diakinesis and metaphase-I. The distribution of the chromosomes at anaphase-I was 10 and 11. The progeny with 30 somatic chromosomes showed an erect growth habit with oblong leaves 11.5 cm. long and 6.9 cm. broad. The chromosome complement consisted of 18 with median and 12 with sub-median primary constrictions and 3 with secondary constrictions.

Examination of three seedlings of the open-pollinated 'Indonesian Seedless' guava showed somatic chromosome numbers of 21, 24, and 25 (Hirano and Nakasone, 1969a).

MATERIALS AND METHODS

Source of Materials

The 'Indonesian Seedless' guava was introduced into Hawaii from India in 1965 by Dr. R. A. Hamilton. Vegetatively propagated plants of this clone were planted at the Poamoho Experimental Station. One plant was grown in a container on the University of Hawaii Manoa campus for detailed observation.

Forty seedlings, obtained from open-pollinated seeds of 'Indonesian Seedless', were planted in 5-gallon cans for morphological and pollination studies. These seedlings were identified by the symbols I.S. for 'Indonesian Seedless', and numbered from 1 to 40.

'J. H. Beaumont', a diploid (Hirano and Nakasone, 1969a) clone, was used as the source of pollen and for comparison of characters.

Chromosome Numbers

Greenwood cuttings of the seedlings were rooted in the mist box after treatment with 2,000 ppm. SNA/talc mixture. Rooted cuttings were grown in containers with vermiculite as a growing medium for easy access to the root tips. An organic fertilizer was applied weekly to the plants.

Root tips for chromosome counts were collected between 11 a.m. and 12 noon and pre-treated in a 0.002M solution of 8-oxyquinoline for 4 hours at about 65^{o} F (Kamemoto, Tanaka, and Kosaki, 1961). They were then prepared according to the method described by Dyer (1963):

> Fix for 5 min. in 10 parts ethyl alcohol: 2 parts acetic acid: 2 parts chloroform: 1 part formalin. Macerate in 1N HCl at 60^{o} C for 5 min. Tap out in lacto-propionic acid for 5 min. Squash under a cover slip.

External Morphological Characters

Characters such as leaf width, leaf length (minus the petiole), petiole length and internode length were measured. The ratio of leaf width/leaf length was determined to provide an idea of the broadness of the leaf. Leaf width, leaf length and petiole length were taken from the first five leaves below the green juvenile stem. The internode length was averaged from the first four to ten internodes below the green juvenile stem. The general character of the leaf was described, especially its shape and its apex.

The general plant growth habit, flowering and fruiting habits were also noted. The size of the flower, width of the ovary and lengths of the style and peduncles were measured. The fruit weight, length, diameter and shape were also recorded.

Pollen Germination

In vitro pollen germination studies were conducted using the method described by Brewbaker and Kwack (1963), with the addition of 1 percent agar. The pH of the medium was adjusted to 5.0 (Hirano and Nakasone, 1969b) using HCl (Kwack, 1965). The flower buds which provided the pollen were bagged a day prior to anthesis to prevent contamination. The nutrient agar medium was set on glass slides and the pollen grains spread on the surface of the medium. They were left in an enclosed container under high humidity for 24 hours at room temperature (approximately 80° F) and then examined for germination. The pollen grains were considered germinated when the pollen tubes were visible.

Other Characters

Internal fruit characters such as flesh color, shell thickness and number of seeds were examined.

When sufficient flowers were available, self-compatibility studies were conducted. The flowers were bagged and tagged the day before anthesis and self-pollinated the following morning. Cross-compatibility studies using 'J. H. Beaumont' pollen were also conducted. The stamens, together with parts of the sepals and petals, were removed from the receptor flower on the day prior to anthesis and the flower was bagged. The donor buds were also bagged the day prior to anthesis and the pollen applied to the stigma on the morning of floral anthesis.

RESULTS

Plants with 22 Chromosomes

Three seedlings were found to have the diploid chromosome number. They were I.S.7 (Fig. 24), I.S.27 (Fig. 25) and I.S.33 (Fig. 26). Although the chromosome number was the same, several distinct morphological differences were observed among them.

The growth habit and leaves (Fig. 49) of I.S.27 resembled more closely that of a normal diploid (Fig. 47) than those of I.S.7 (Fig. 48) or I.S.33 (Fig. 50).

I.S.7 (Fig. 1) was more compact with denser foliage, smaller leaves and shorter petioles and internodes than the other diploid seedlings (Table I). I.S.27 was upright (Fig. 4) and I.S.33 spreading (Fig. 2).

The petiole of I.S.33 was distinctly longer than those of the others and the leaves were lighter green and slightly pandurate.

The leaf apices of all the plants were acute or slightly accuminate.

I.S.33 flowered heavily. I.S.27 produced some flowers but I.S.7 did not produce any flowers.

Guava plants normally have single axillary flowers, but may produce some 2- or 3-flowered cymes as well as more than one peduncle per axil. Such 2- or 3-flowered cymes were sometimes observed on I.S.33.

Flowers of I.S.33 had more petals (Table II) and narrower petals (Fig. 18) than those of a normal diploid plant. It also had smaller flowers, shorter styles and exhibited some degree of petalody of stamens. The petals of I.S.27 (Fig. 17) were slightly rounder than those of a normal diploid (Fig. 16).

The pollen germination percentage of the plants was lower than that of 'J. H. Beaumont' (Table III). The normal diploid gave 84.4 percent germination while the I.S. diploids, I.S.27 and I.S.33, gave 64.0 and 51.0 percent respectively.

Self-pollinations of I.S.33 did not result in fruit set but crossing with 'J. H. Beaumont' did produce a low percentage of fruit set (Table III).

Open-pollinated fruit set was good on I.S.33. The fruits were small, white-fleshed and globose to ovate in shape (Table IV). Skin was smooth and the fruits often showed blossom-end rot.

Plants with 23 and 24 Chromosomes

I.S.22 and I.S.3 were found to have 23 (Fig. 27) and 24 (Fig. 28) chromosomes, respectively. I.S.3 (Fig. 7 and 52) resembled a normal diploid more closely in vegetative characters because of its larger leaves, and longer petioles and internodes (Table I) when compared with I.S.22 (Fig. 5 and 51).

In reproductive characters, I.S.22 resembled more closely a normal diploid than did I.S.3. Flower production of I.S.22 was heavier than of I.S.3. In both cases flowers were generally borne singly, but in I.S.22 some flowers were produced in cymes. Flowers of I.S.22 (Fig. 19) tended to have more petals, wider ovaries, longer styles and longer peduncles (Table II) than those of I.S.3 (Fig. 20). Petalody of stamens was observed in flowers of both plants.

The 98.4 percent pollen germination of I.S.22 was higher than that of 'J. H. Beaumont' (Table III). I.S.3 showed 57.6 percent pollen germination.

Both cross- and self-pollinations resulted in fruit set in I.S.22. Relatively large open-pollinated fruits were obtained from both I.S.22 and I.S.3.

The fruits of I.S.22 were pink-fleshed, globose to slightly ovate in shape, and contained numerous small seeds (Table IV). Cross- and open-pollination fruits of I.S.22 contained twice as many seeds as the selfed fruits. The fruits of I.S.3 were white-fleshed, pear-shaped and had less seeds.

Plants with 25 Chromosomes

Three seedlings were found to possess 25 chromosomes. They were I.S.5 (Fig. 29), I.S.8 (Fig. 30), and I.S.19 (Fig. 31).

The plants in this group differ widely in morphological characters except that their leaves are relatively small and broad and their petioles short (Table I). They did not resemble the normal diploids nor the triploids. The leaves of I.S.5 (Fig. 53) were thick and curled with acute, slightly accuminate, or obtuse apices. The leaves of I.S.8 were acute or slightly accuminate at the apices. Leaves of guava plants are generally opposite, but in I.S.8, another leaf may appear at one axil, making it irregularly 3-whorled. The leaves of I.S. 19 were slightly pandurate (Fig. 39).

The growth habit of I.S.8 (Fig. 8) was not as spreading and branching as that of I.S.5 (Fig. 3) or I.S.19 (Fig. 10).

No flowers were produced by I.S.5 or I.S.8. However, I.S.19 produced flowers which were not very different from those of a normal diploid (Fig. 21), except that the ovary was narrower, the style shorter, and the peduncles longer (Table II). Flowers were generally borne singly, but two peduncles were observed arising from the same axil, each containing one or two flower buds. Petalody of stamens was present.

Pollen germination of I.S.19 was 52.5 percent, which was even lower than that of I.S.3 (Table III). Open-pollinated fruits were small, slightly ovate in shape, orange-pink fleshed and contained few seeds (Table IV).

Plants with 28, 31, and 32 Chromosomes

Chromosome numbers of 28, 31 and 32 were found for I.S.1 (Fig. 32), I.S.4 (Fig. 33) and I.S.10 (Fig. 34), respectively. The plants in this group had a relatively compact growth habit. This was especially true of I.S.1 (Fig. 9) and I.S.10 (Fig. 12). The plant of I.S.4 is shown in Figure 11.

Leaf characters varied (Table I). I.S.10 (Fig. 42) had the smallest leaf, the shortest petiole, and the shortest internode in the group; I.S.4 (Fig. 41) had the largest leaf and longest internode of the three. The leaf of I.S.1 is shown in Figure 40.

I.S.10 did not produce any flowers, but I.S.1 and I.S.4 produced flowers, usually singly borne, but sometimes from 2 peduncles arising at the same leaf axil. The petals of I.S.4 often did not split open at anthesis (Fig. 22) and remained as a cap or else fell off intact.

Pollen of I.S.1 showed a low percent germination (Table III) and both self- and cross-pollination failed to set fruit. An attempt at self-pollination of I.S.4 also did not produce fruit set.

Plants with 33 Chromosomes

Three seedlings were found to have the triploid number of chromosomes. They were I.S.25 (Fig. 35), I.S.36 (Fig. 36) and I.S.39 (Fig. 37). I.S.25 (Fig. 6) had an erect and branching growth habit. I.S.36 (Fig. 14) and I.S.39 (Fig. 15) were relatively young plants, but possessed the erect and branching habit somewhat resembling that of the 'Indonesian Seedless' parent (Fig. 13).

In the triploid plants, the leaves (Fig. 43, 44, and 45) were relatively large and broad (Table I) but were not as large as those of the 'Indonesian Seedless' parent (Fig. 46). Petiole and internode lengths of the parental clone were longer than those of the three triploid seedlings.

The leaf apices differed slightly among the plants of the group. Those of I.S.36 were generally obtuse and sometimes acute. Those of I.S.39 were acute or slightly accuminate, while the leaf apices of I.S.25 varied from slightly accuminate to obtuse.

No flowers were produced by any of the three triploid seedlings. The triploid 'Indonesian Seedless' flower is shown in Figure 23.

TABLE I. LEAF CHARACTERS AND INTERNODE LENGTHS OF GUAVA PROGENY STUDIED

Plant	Chromosome number	Leaf (cm.)[a]		W/L ratio	Length (cm.)	
		Width (W)	Length (L)		Petiole[a]	Internode[b]
J. H. Beaumont	22	6.5	11.7	0.55	0.6	4.4
I.S.7	22	3.1	6.6	0.47	0.4	2.2
I.S.27	22	5.6	10.7	0.53	0.4	3.2
I.S.33	22	3.7	7.5	0.49	0.7	2.8
I.S.22	23	3.6	8.7	0.42	0.3	3.0
I.S.3	24	5.3	11.2	0.47	0.8	4.2
I.S.5	25	5.1	8.7	0.59	0.2	2.8
I.S.8	25	4.6	7.2	0.64	0.2	2.3
I.S.19	25	3.6	7.0	0.51	0.4	2.9
I.S.1	28	4.6	8.1	0.57	0.5	2.4
I.S.4	31	6.8	10.0	0.68	0.4	3.1
I.S.10	32	3.5	6.0	0.59	0.3	1.8
I.S.25	33	6.1	10.3	0.59	0.5	2.9
I.S.36	33	6.5	9.9	0.65	0.3	3.1
I.S.39	33	5.2	11.6	0.45	0.4	3.5
Indonesian Seedless	33	7.7	14.1	0.54	0.7	4.8

[a] Average of 5 leaves below the green juvenile stem.
[b] Average of 4 to 10 internodes below the green juvenile stem.

TABLE II. FLORAL MORPHOLOGY OF GUAVA PROGENY STUDIED

Plant	Chromosome number	Number of flowers measured	Average Number of petals	Petalody of stamens	Average Width (cm.)		Average Length (cm.)	
					Flowers	Ovary	Style	Peduncle
J. H. Beaumont	22	13	4.8	Absent	3.4	0.42	1.04	1.2
I.S.33	22	20	7.2	Present	3.0	0.39	0.80	1.3
I.S.22	23	2	5.0	Present	3.4	0.45	1.10	2.3
I.S.19	25	8	4.3	Present	3.5	0.39	0.71	1.7

TABLE III. POLLEN GERMINATION AND COMPATIBILITY STUDIES

Plant	Chromosome number	Percent pollen germination[a]	Confidence interval (95%)	Compatibility (Fruit sets/Attempts)	
				Selfed	Crossed[b]
J. H. Beaumont	22	84.4	79-89	--	--
I.S.27	22	64.0	58-70	--	--
I.S.33	22	51.0	46-58	0/13	1/3
I.S.22	23	98.4	95-99	2/4	3/4
I.S.3	24	57.6	52-64	--	--
I.S.19	25	52.5	48-60	--	--
I.S.1	28	15.1	10-20	0/1	0/1
I.S.4	31	--	--	0/1	--
Indonesian Seedless	33	1.1	0-4	0/10	0/8

[a] Based on sample size of 250 pollen grains.
[b] Pollen from diploid 'J. H. Beaumont' clone.

TABLE IV. FRUIT CHARACTERISTICS OF GUAVA PROGENY STUDIED

Plant	I.S.33	I.S.22			I.S.3	I.S.19	Indonesian Seedless
Chromosome number	22	23			24	25	33
Cross	Open-pollinated	X J. H. Beaumont	Selfed	Open-pollinated	Open-pollinated	Open-pollinated	Open-pollinated
Number of fruits averaged	4	1	2	6	6	1	5
Weight per fruit (gm.)	22.5	59.4	45.9	66.1	70.9	11.8	89.8
Diameter (D cm.)	3.3	4.7	4.3	4.7	4.7	2.8	5.7
Length (L cm.)	3.4	4.9	4.4	5.2	6.2	2.6	4.9
Ratio D/L	0.98	0.96	0.98	0.90	0.76	1.06	1.17
Shape	Round to obovate	Ovate	Round to oval	Slightly ovate	Pyriform	Slightly ovate	Flattened endwise
Flesh color	White	Pink	Pink	Pink	White	Orange-pink	White
Shell thickness (cm.)	0.6	0.8	0.8	0.8	0.9	0.6	1.3
Number of seeds	116	214	143	263	128	20	42

PLATE I*

FIGURE 1. PLANT OF I.S. 7 (22 CHROMOSOMES)

FIGURE 2. PLANT OF I.S. 33 (22 CHROMOSOMES)

FIGURE 3. PLANT OF I.S. 5 (25 CHROMOSOMES)

*Scale given in photographs (one mark = 1 ft.)


1
2
3

PLATE II*

FIGURE 4. PLANT OF I.S. 27 (22 CHROMOSOMES)

FIGURE 5. PLANT OF I.S. 22 (23 CHROMOSOMES)

FIGURE 6. PLANT OF I.S. 25 (33 CHROMOSOMES)

FIGURE 7. PLANT OF I.S. 3 (24 CHROMOSOMES)

*Scale given in photographs (one mark = 1 ft.)


4


5


6


7

PLATE III*

FIGURE 8. PLANT OF I.S. 8 (25 CHROMOSOMES)

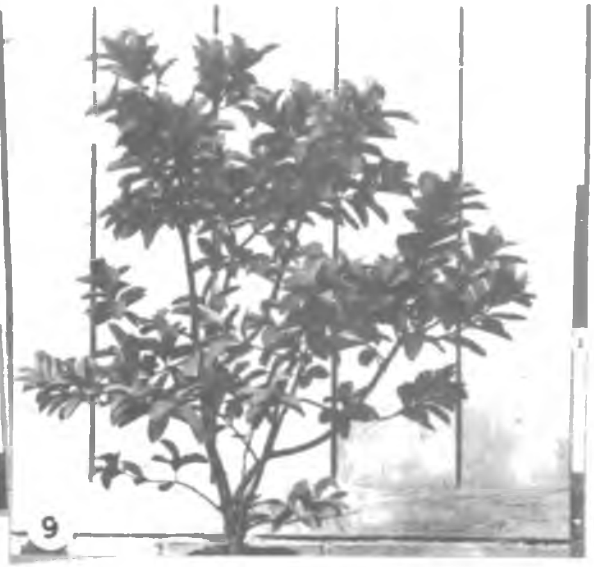
FIGURE 9. PLANT OF I.S. 1 (28 CHROMOSOMES)

FIGURE 10. PLANT OF I.S. 19 (25 CHROMOSOMES)

FIGURE 11. PLANT OF I.S. 4 (31 CHROMOSOMES)

*Scale given in photographs (one mark = 1 ft.)

8



9

10

11

PLATE IV*

FIGURE 12. PLANT OF I.S. 10 (32 CHROMOSOMES)

FIGURE 13. PLANT OF TRIPLOID 'INDONESIAN SEEDLESS' CLONE

FIGURE 14. PLANT OF I.S. 36 (33 CHROMOSOMES)

FIGURE 15. PLANT OF I.S. 39 (33 CHROMOSOMES)

*Scale given in photographs (one mark = 1 ft.)


12


13

14


15

PLATE V*

FIGURE 16. FLOWER OR DIPLOID 'J. H. BEAUMONT' CLONE

FIGURE 17. FLOWER OF I.S. 27 (22 CHROMOSOMES)

FIGURE 18. FLOWER OF I.S. 33 (22 CHROMOSOMES)

FIGURE 19. FLOWER OF I.S. 22 (23 CHROMOSOMES)

FIGURE 20. FLOWER OF I.S. 3 (24 CHROMOSOMES)

FIGURE 21. FLOWER OF I.S. 19 (25 CHROMOSOMES)

*All figures on same scale.

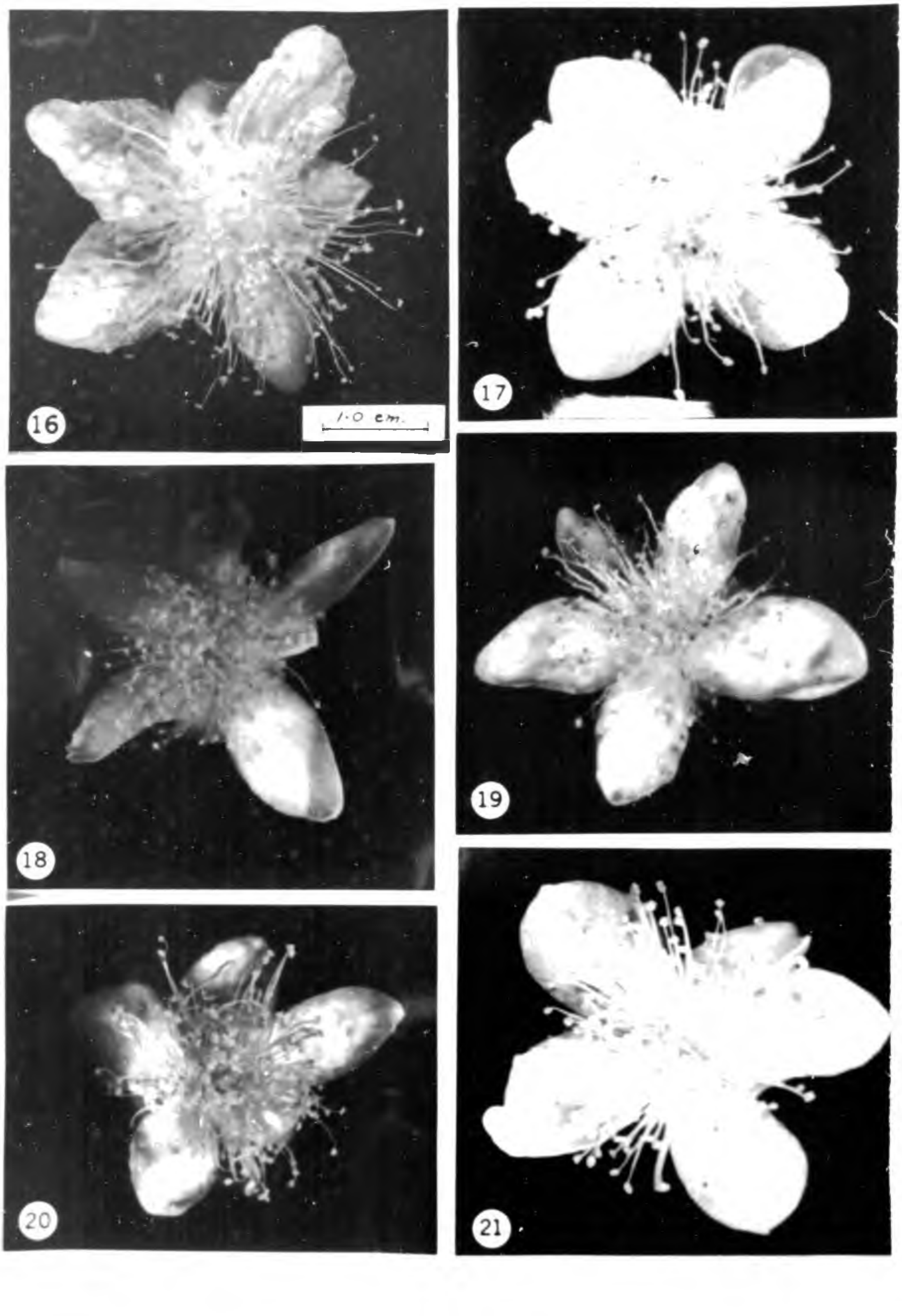

16
1.0 cm.
17
18
19
20
21

PLATE VI

FIGURE 22. FLOWER OF I.S. 4 (31 CHROMOSOMES)*

FIGURE 23. FLOWER OF TRIPLOID 'INDONESIAN SEEDLESS' CLONE*

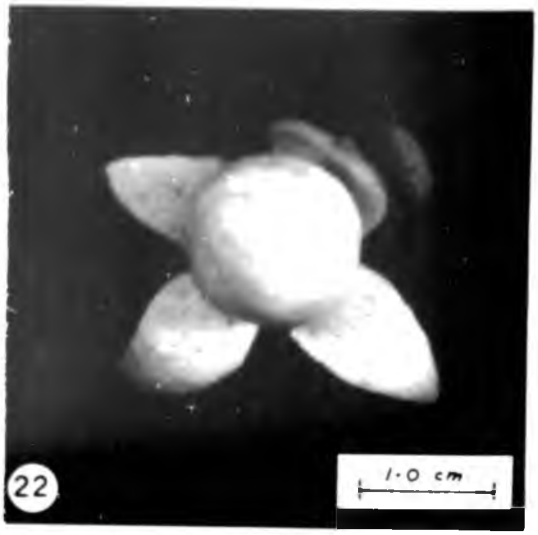
FIGURE 24. 22 CHROMOSOMES OF I.S. 7

FIGURE 25. 22 CHROMOSOMES OF I.S. 27

FIGURE 26. 22 CHROMOSOMES OF I.S. 33

FIGURE 27. 23 CHROMOSOMES OF I.S. 22

*Figures on same scale.



22

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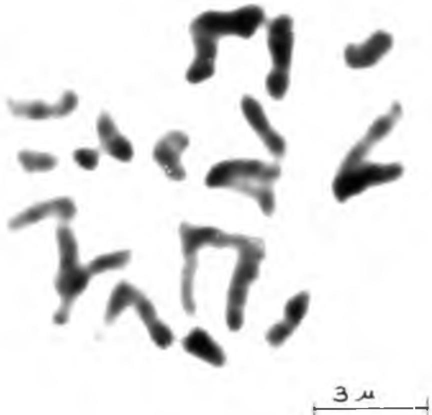
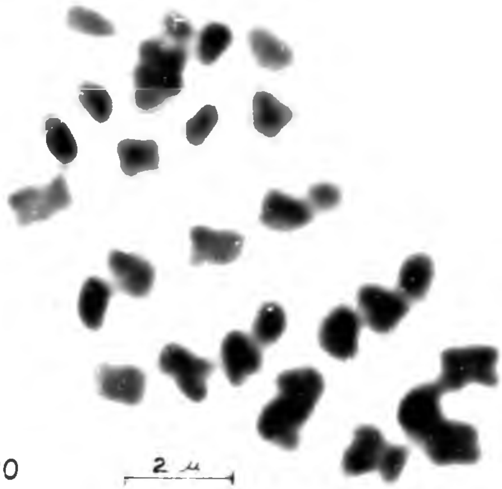
PLATE VII

FIGURE 28.	24 CHROMOSOMES OF I.S. 3
FIGURE 29.	25 CHROMOSOMES OF I.S. 5
FIGURE 30.	25 CHROMOSOMES OF I.S. 8
FIGURE 31.	25 CHROMOSOMES OF I.S. 19
FIGURE 32.	28 CHROMOSOMES OF I.S. 1
FIGURE 33.	31 CHROMOSOMES OF I.S. 4



28

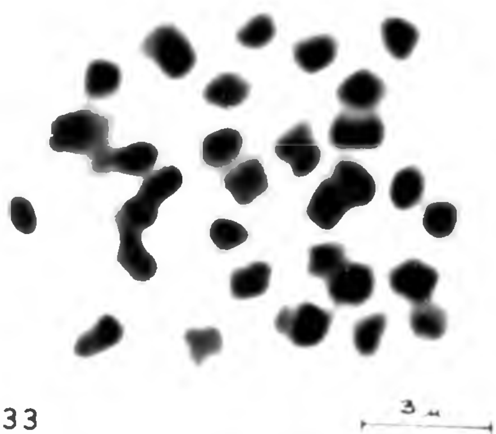
29



30

31

32



33

PLATE VIII

FIGURE 34. 32 CHROMOSOMES OF I.S. 10

FIGURE 35. 33 CHROMOSOMES OF I.S. 25

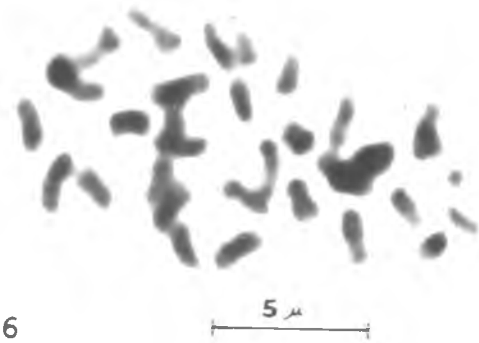
FIGURE 36. 33 CHROMOSOMES OF I.S. 36

FIGURE 37. 33 CHROMOSOMES OF I.S. 39

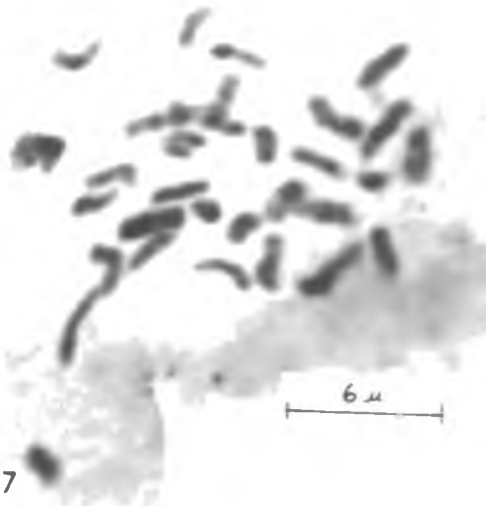
FIGURE 38. I.S. 30: A WEAK PLANT

34

35



36



37

38

PLATE IX*

FIGURE 39. LEAF OF I.S. 19 (25 CHROMOSOMES)

FIGURE 40. LEAF OF I.S. 1 (28 CHROMOSOMES)

FIGURE 41. LEAF OF I.S. 4 (31 CHROMOSOMES)

FIGURE 42. LEAF OF I.S. 10 (32 CHROMOSOMES)

FIGURE 43. LEAF OF I.S. 25 (33 CHROMOSOMES)

FIGURE 44. LEAF OF I.S. 36 (33 CHROMOSOMES)

FIGURE 45. LEAF OF I.S. 39 (33 CHROMOSOMES)

FIGURE 46. LEAF OF TRIPLOID 'INDONESIAN SEEDLESS' CLONE

*All figures on same scale as shown in Fig. 39.

39

5 cm

40

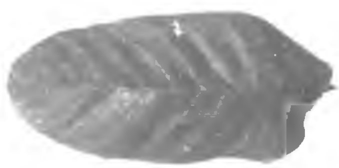


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PLATE X*

FIGURE 47. LEAF OF DIPLOID 'J. H. BEAUMONT' CLONE

FIGURE 48. LEAF OF I.S. 7 (22 CHROMOSOMES)

FIGURE 49. LEAF OF I.S. 27 (22 CHROMOSOMES)

FIGURE 50. LEAF OF I.S. 33 (22 CHROMOSOMES)

FIGURE 51. LEAF OF I.S. 22 (23 CHROMOSOMES)

FIGURE 52. LEAF OF I.S. 3 (24 CHROMOSOMES)

FIGURE 53. LEAF OF I.S. 5 (25 CHROMOSOMES)

FIGURE 54. LEAF OF I.S. 8 (25 CHROMOSOMES)

*All figures on same scale as shown in Fig. 47.

47

5 cm

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53

54

DISCUSSION

Chromosome counts of the open-pollinated seedlings derived from a triploid guava clone support the suggestion of Hirano and Nakasone (1969a) that the aneuploid series resulted from a triploid X diploid cross. A similar series was reported by D'Cruz and Rao (1962) in a progeny of a seedless triploid guava plant.

The chances of the triploid guava fertilizing itself are remote because of its poor pollen viability (Table III) and the tendency towards complete seedlessness when grown by itself (Whitman, 1962). Hirano and Nakasone (1969b) also showed poor pollen viability in the triploid 'Indonesian Seedless'.

The possibilities of apomictic seed development in the triploid, as suggested by D'Cruz and Rao (1962), could be ruled out as none of the seedlings with 33 chromosomes closely resembled the seed parent (Table I, Fig. 6, 13-15, 43-46). This further supports the occurrence of cross-pollination.

Variations in morphological characters occur between individuals with different chromosome numbers and also between individuals with the same chromosome number. An exception was that plants with 25 chromosomes appeared to have smaller leaves than plants with 33 chromosomes (Table I, Fig. 53, 54, 39, 43-45). However, unless accompanied by karyotype studies, it would not be possible to relate morphological changes to variations in their chromosome complements. This could be complicated by the fact that the progeny studied was the product of open-pollination. Variations in fruit characters, as in the white-fleshed pyriform fruits of I.S.3, and the pink-fleshed, round to slightly ovate fruits of I.S.22, suggest pollination from several different sources.

The high percentage of pollen germination obtained in the plant with 23 chromosomes, the reduced but still relatively good pollen germination in plants with 24, 25, and 28 chromosomes, and the fruit set on the 23-, 24-, and 25-chromosome plants all suggest that the guava plant has some tolerance to unbalance in chromosome number.

The relatively low pollen germination of 64.0 percent and 51.0 percent in the two diploid seedlings when compared to the 84.4 percent pollen germination in 'J. H. Beaumont' probably indicates that these diploid seedlings do not have 11 normal homologous pairs of chromosomes.

The difference in the pollen germination percentage of 'J. H. Beaumont' obtained here and the 99.3 percent obtained by Hirano and Nakasone (1969b) could be due to slight differences in the methods used or to the effects of different environmental conditions. Pollen germination percentages have been found to vary greatly even among established guava clones (Balasubrahmanyam, 1959; D'Cruz and Rao, 1962; Nair, Balasubrahmanyam, and Khan, 1964; Hirano and Nakasone, 1969b). Zimmerman (1968) found that pollen grain sterility in *Rhoeo spathacea* was caused by internal factors such as disharmonic combinations of chromosomes in the early stages of grain development, while at the later stages, pollen sterility depended extensively upon environmental conditions.

The question of incompatibility arises when 13 attempts at self-pollination in one diploid seedling did not result in any fruit set while 3 attempts at cross-pollination using 'J. H. Beaumont' pollen gave one fruit set (Table III). Cross- and open-pollinated fruits of I.S.22

contained almost twice as many seeds as selfed fruits (Table IV). Among commercial clones, some degree of self-incompatibility could account for the low (27 percent) fruit set in the 'Lucknow' guava (Ito and Nakasone, 1968). In the same study, clonal incompatibility was observed in crosses involving the triploid 'Indonesian Seedless' as a female parent. No fruit set was obtained with 'J. H. Beaumont' pollen but 57 percent fruit set was obtained with pollen from clone No. 7197. Clonal cross-incompatibility, including reciprocal incompatibility, has been reported among some Indian clones (Seth, 1960). Further examination by Seth indicated that incompatibility was of the gametophytic type in which pollen tube growth was inhibited in the style.

Chromosome numbers of the progeny of the 3n X 2n *Psidium guajava* cross counted in this study ranged from 2n to 3n. Similar results had been reported previously. Hirano and Nakasone (1969a) reported chromosome numbers of 21, 24 and 25 for three plants of the same progeny. D'Cruz and Rao (1962) reported chromosome numbers of 21 and 30 for two seedlings of a similar cross involving another triploid guava plant. All the plants examined cytologically in this study were rooted greenwood cuttings. Several weak plants (Fig. 38), which did not produce greenwood rooting materials or which failed to root, could possibly have chromosome numbers which fall above or below the range reported. In *Gladiolus*, the progeny of a 3n X 2n cross had chromosome numbers ranging from 33 to 75, with the majority of the plants being either triploids (3n=45) or tetraploids (4n=60) (Jones and Bamford, 1942). However, examination of 285 plants of a progeny of a 3n X 2n *Datura stramonium* cross showed a different distribution pattern.

20.4 percent were found to be diploid, 48.4 percent 2n+1, 27.7 percent 2n+1+1, and 3.5 percent 2n+1+1+1 (Avery, Satina, and Rietsema, 1959).

Guava plants with chromosome numbers of 23, 28, 31, and 32 are being reported for the first time and this is also the first evidence of recovery of diploid and triploid guava plants from a triploid seed parent.

SUMMARY

1. Chromosome counts were made of 14 seedlings derived from open-pollination of the triploid 'Indonesian Seedless' guava clone and their characters described.
2. Three seedlings had 22 chromosomes, one had 23, one 24, three had 25, one 28, one 31, one 32, and three 33 chromosomes.
3. Plants with chromosome numbers of 23, 28, 31, 32 and the recovery of diploid and triploid guava plants from a triploid seed parent are reported for the first time.
4. Variations in morphological characters were found to occur between individuals with different chromosome numbers and also between individuals with the same chromosome number.
5. The results suggest that the seedlings were derived from a cross between the triploid seed parent and several diploid sources.
6. Pollen germination studies showed that the guava plant tolerates some degree of chromosomal unbalance.
7. Some degree of self-incompatibility could account for the low fruit set or low number of seeds formed from self-pollination.

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